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Technical note

FTMS and TOF/TOF mass spectrometry in concert: Identifying peptides with high reliability using matrix prespotted MALDI target plates☆

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Abstract

In this paper we describe a combination of the mass spectrometric techniques MALDI–TOF/TOF and MALDI–FTMS to identify proteins in complex samples using prespotted MALDI target plates. By this procedure accurate FTMS mass measurements and TOF/TOF data are obtained from the same spot. We have found that this combination of techniques leads to more reliable identification of peptides.

Keywords: Proteomics; FTMS; MALDI–TOF/TOF; Peptide identification

MALDI–TOF mass spectrometry is an attractive technique for peptide profiling for reasons of its sensitivity, reliability, and high throughput capability [1]. However, the extreme complexity of peptide mixtures derived from proteins as well as the large dynamic range of the abundances of proteins in body fluids, tissue and cell lysates makes it all but impossible to detect all tryptic peptides from a sole mass spectrum. In addition, TOF peptide profiles are often difficult to interpret, mainly because a monoisotopic peak of one peptide may overlap with an isotopic peak of another, a feature the peak-picking algorithm may not detect. Higher mass resolution and mass accuracy as supplied, for example, by FTMS may alleviate some of these drawbacks. These matters are exemplified in Fig. 1, which shows a partial MALDI–TOF (panel A) and MALDI–FTMS (panel B) mass spectrum of a trypsinized (albumin depleted) serum sample: the monoisotopic peaks of the two peptides can be easily identified from the FTMS mass spectrum and the exact masses are then obtained, as indicated. Fig. 1 illustrates another major advantage of MALDI–FTMS over MALDI–TOF, namely that the FTMS spectra, and in contrast to the MALDI–TOF spectra, hardly contain signals often referred to as "chemical noise" [2]. Thus, the signal to noise ratio in FTMS is much larger (134 for FT) than that for TOF data (14 for TOF). Using FTMS, mass measurement with low ppm accuracies are now routinely obtained. Using judiciously chosen calibration procedures accuracies below 0.5 ppm can be obtained on a Bruker Daltonics Apex Q 9.4 Tesla instrument. In addition, the FTMS technique offers sensitivity in the femtomole range in complex samples [3].

However, the identification of peptides from a mass measurement alone requires a mass accuracy below 0.1 ppm which is not yet possible with most FTMS mass spectrometers [4]. Also the sensitivity and selectivity of MS/MS experiments on MALDI ions in FTMS by collision-induced dissociation (CID) for complex peptide mixtures is still not on a par with multiply charged peptides generated by electrospray ionization.

We have therefore developed a simple method for the identification of peptides in complex mixtures whereby the high mass accuracy and resolution of MALDI–FTMS is exploited for directing and confirmation purposes. First, from MALDI–FTMS peptide profiling experiments peaks are identified that show a significant difference in expression between the control and patient group. Next, a fractionation was performed using a C18 Pep Map column (75 μm i.d. × 150 mm, 3 μm, Dionex, Sunnyvale, CA, USA) on a nanoscale liquid chromatography system (nanoLC) (Dionex, Sunnyvale, CA, USA). Five microliters of the sample was loaded onto the trap column (300 μm i.d. × 5 mm, 5 μm, Dionex, Sunnyvale, CA, USA). Fractionation was performed using a 130 min gradient from 0 to 76% of acetonitrile, ACN, (solution A (100% H_2O, 0.05% TFA) and solution B (80% ACN, 20% H_2O and 0.04%

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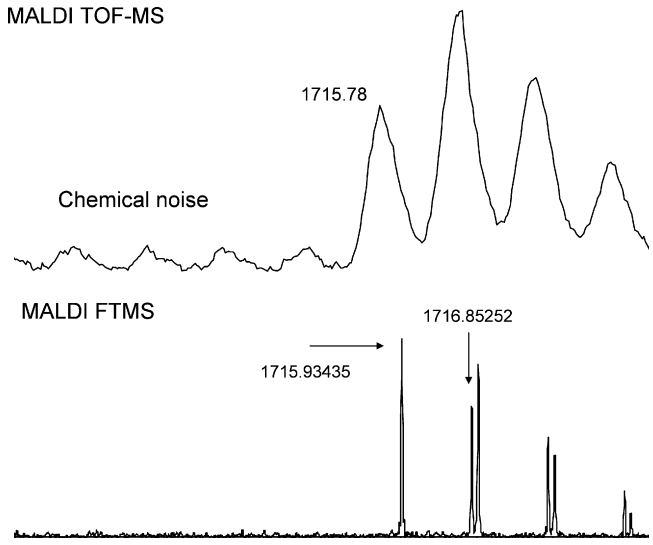

Fig. 1. Partial MALDI–TOF and MALDI–FT mass spectra of a tryptic digest of proteins of an albumin depleted serum sample. Monoisotopic peaks of the two peptides can be easily identified from the FTMS mass spectrum, because of the superior resolution compared to the MALDI–TOF measurement. In addition the chemical noise, present in the MALDI–TOF spectrum, is absent in the MALDI–FT mass spectrum.

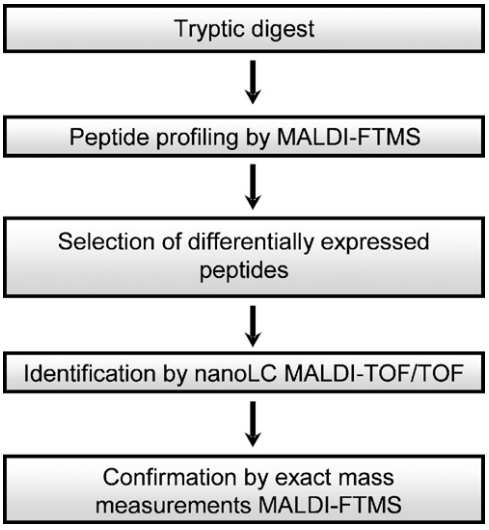

Fig. 2. Flowchart of the identification procedure.

TFA); 0–15 min, 0% solution B, 15.1 min 15%, 75 min 40%, 90 min 70%, 90.1–100 min 95%, 100.1 min 0% and 130 min 0%). Fifteen-second fractions were spotted automatically onto a commercially available prespotted MALDI plate containing 384 spots (Bruker Daltonics, USA) covered with α-cyano-4-hydroxycinnamic acid (HCCA) matrix, using a robotic system (Probot Micro Fraction Collector, Dionex, Sunnyvale, CA, USA). To each fraction, 1 μl water was added. Finally, salts were removed by washing the prespotted plate for 5 s with a 10 mM $(NH_4)H_2PO_4$ solution in 0.1% TFA/water solution. The spots were subsequently measured by automated MALDI–TOF/TOF (Ultraflex, Bruker Daltonics, Germany using WARLP-LC software (Bruker Daltonics, Germany)). By this procedure MS

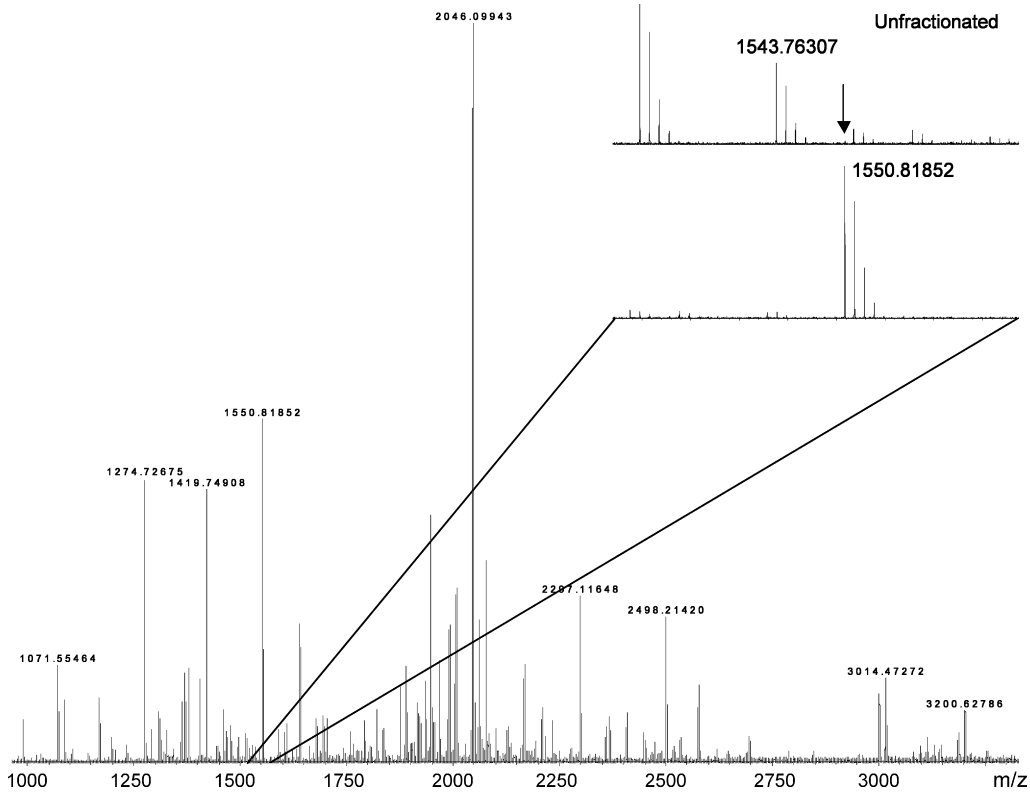

Fig. 3. MALDI–FT mass spectrum of a nanoLC fraction of a frozen section of trypsynized placenta material. This fraction contains as many peaks as the unfractionated material. The upper part of the inset shows part of the mass spectrum obtained from the unfractionated sample. Even the very small peak indicated by the arrow gives after separation an intense signal.

spectra of each individual spot was obtained and subsequently MS/MS experiments are performed on each peptide. The best spots for performing the MS/MS measurements were determined automatically by the WARLP-LC software.

The idea was to use this prespotted plate also for MALDI–FTMS experiments to confirm, by exact mass measurement, the identity of the differentially expressed peptides. Unfortunately on our Bruker Apex Q 9.4 Tesla FTMS, the matrix HCCA produces only very weak signals in MALDI–FTMS experiments. However, when after the TOF/TOF data have been acquired, the spot of interest is covered with 0.5 μl of a DHB solution (10 mg/ml 2,5-dihydroxy benzoic acid in 0.1% TFA) and then introduced into the FTMS, intense spectra are obtained allowing FTMS experiments on the same spot as was used to acquire the TOF/TOF data. The workflow of this procedure is shown in Fig. 2. Confirmation proceeds via two paths: the exact mass measured in the final step is compared to the calculated mass of the identified peptide and to the exact mass measured in the peptide profiling experiment.

An example of our procedure is presented in Fig. 3 which shows the full MALDI–FTMS mass spectrum of a nanoLC fraction of a frozen section of trypsinized placenta material [5]. This spectrum, following the workflow of Fig. 2, was obtained after the TOF/TOF measurements. This spectrum contained as many peaks as the MALDI–FTMS mass spectrum of the original sample (i.e. prior to LC separation). In the inset is given a small part of the spectrum of the original sample (panel A) and of the above fraction (panel B). It can be seen that even almost undetectable signals in the original spectrum can, after LC separation, be measured with great precision.

Finally, the prespotted plate with the deposited peptide mixtures can be stored in a dark environment at room temperature for at least one month without significant loss of signal intensity, allowing remeasurement when required at a later stage. We have applied our procedure successfully to various projects and the results will be published in separate papers.

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